SUPPLEMENTARY METHODS

Animals and cell lines. Gt(ROSA)26Sor$^{tm1(Luc)Kaelin11}$ (Rosa26-Fluc), B6;129-Gt(ROSA)26Sor^{tm1Joe}/J[27] (Rosa26-GNZ), B6;129S2-Scarb1^{tm1Kri}/J[17] (SCARB1-/-) and FVB/NJ (wild-type) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Rosa26-Fluc mice contain the firefly luciferase (luc) gene inserted into the Gt(ROSA)26Sor locus. Expression of the luciferase gene is blocked by a loxP-flanked STOP fragment placed between the luc sequence and the Gt(ROSA)26Sor promoter. CRE recombinase mediated excision of the transcriptional stop cassette results in luciferase expression in Cre-expressing tissues. Rosa26-GNZ knock-in mice have widespread expression of a nuclear-localized green fluorescent protein/beta-galactosidase fusion protein (GFP-NLS-GNZ) once an upstream loxP-flanked STOP sequence is removed. When CRE recombinase is introduced into cells the resulting GNZ fusion protein expression allows for enhanced (single cell level) visualization. Mice were bred and maintained at the Comparative Bioscience Center of the Rockefeller University according to guidelines established by the Institutional Animal Committee. Huh-7.5, Huh-7.5.1, 293T, HepG2 and HEK293 were maintained in DMEM with 10% fetal bovine serum (FBS) and 1% nonessential amino acids (NEAA) and 786-O cells were maintained in RPMI with 10% FBS and 1% NEAA.

Antibodies

Blocking antibodies against CD81 (JS81) and IgG1 control antibodies were obtained from BD Biosciences (Franklin Lakes, NJ). Antibodies against NS5A[28] and E2 (clone 3/11)[29] have been described previously. Antibodies for the detection of human CD81 were purchased from BD Biosciences, OCLN from BD Biosciences (for histology) and from Invitrogen (Carlsbad, CA, for Western blotting) CLDN1 from Invitrogen (for Western blotting) and Abcam (Cambridge, MA for histology) and SCARB1 from Genetex (Irvine, CA, for histology) and from BD Biosciences (for Western blotting). Antibodies for *in vivo* depletion of T cells (anti-mouse CD4 and anti-mouse CD8) were obtained from Biolegend (San Diego, CA) and the antibody for depletion of NK cells (anti-mouse Asialo GM1) was purchased from Cedarlane (Burlington, Ontario, Canada). Fluorochrome-conjugated antibodies against mouse CD3, CD4, CD8, CD49b, Ly-6G were obtained from BD Biosciences and the IRDye700-conjugated anti-Luciferase antibody was acquired from Acris antibodies (San Diego, CA) and Bethyl Laboratories (Montgomery, TX).

Adenovirus constructs

Adenoviral constructs encoding human and murine homologues of the four HCV entry factors (CD81, SCARB1, CLDN1 and OCLN) and fluorescently tagged human SCARB1, CLDN1 and OCLN were created using the AdEasy™ Adenoviral Vector System (Agilent Technologies, Santa Clara, CA) according to the manufacturer's instructions. Briefly, human and murine entry factor cDNA was PCR-amplified from TRIP-based constructs[4] and inserted into the pShuttle-CMV™ using KpnI/XhoI sites (KpnI/NotI for hSCARB1). mKate-SCARB1, Cerulean-CLDN1, and Venus-OCLN fusions were cut from existing TRIP-based constructs and inserted into pShuttle-CMV™ using compatible restriction sites. Recombinant pShuttle-CMV plasmids were linearized with PmeI and ligated to pAdEasy™ by homologous recombination followed by electroporation into BJ5183 cells (Agilent). Recombinant pShuttle-pAdEasy constructs were identified by PacI restriction analysis. All plasmid constructs were verified by DNA sequencing.

Gene		Primer sequence
hCD81	+*	CGCGGTACCCCACCATGGGAGTGGAGGGCTGCAC
	-	ACGCTCGAGTCAGTACACGGAGCTGTTCC
mCD81	+	GCATACAGGGTACCGCCACCATGGGAGTGGAGGGCTGCACCAAA

	-	GCATACAGCTCGAGTCAGTACACGGAGCTGTTCCGGAT
hSCARB1	+	CGCGGTACCCCACCATGGGCTGCTCCGCCAAAGC
	-	CAGCGCTGCGGCCGCCTACAGTTTTGCTTCCTGCA
mSCARB1	+	GCATACAGGGTACCGCCACCATGGGCGGCAGCTCCAGGGCGCGC
	-	GCATACAGCTCGAGCTATAGCTTGGCTTCTTGCAGCAC
hCLDN1	+	CGCGGTACCCCACCATGGCCAACGCGGGGCTGCA
	-	ACGCTCGAGTCACACGTAGTCTTTCCCGC
mCLDN	+	GCATACAGGGTACCGCCACCATGGCCAACGCGGGGCTGCAGCTG
	-	GCATACAGCTCGAGTCACACATAGTCTTTCCCACTAGA
hOCLN	+	CGCGGTACCCCACCATGTCATCCAGGCCTCTTGA
	-	ACGCTCGAGCTATGTTTTCTGTCTATCAT
mOCLN	+	GCATACAGGGTACCGCCACCATGTCCGTGAGGCCTTTTGAAAGT
	-	GCATACAGCTCGAGCTAAGGTTTCCGTCTGTCATAATC

* (+) forward primer; (-) reverse primer

Production of recombinant adenoviruses

Adenoviral stocks were generated as previously described[30]. Briefly, adenoviral constructs were transfected into HEK293 cells (American Type Culture Collection, Manassas, VA) using the calcium-phosphate method. Transfected cultures were maintained until cells exhibited full cytopathic effect (CPE), then harvested and freeze-thawed. Supernatants were serially passaged two more times with harvest at full CPE and freeze-thaw. For virus purification, cell pellets were resuspended in 0.01M sodium phosphate buffer pH7.2 and lysed in 5% sodium-deoxycholate, followed by DNAse I digestion. Lysates were centrifuged and the supernatant was layered onto a 1.2-1.46 g/ml CsCl gradient, then spun at 23,000 rpm on a Beckman Optima 100K-Ultra centrifuge using an SW28 spinning-bucket rotor (Beckman-Coulter, Inc.). Adenovirus bands were isolated and further purified on a second CsCl gradient using an SW41.Ti spinning-bucket rotor. Resulting purified adenoviral bands were isolated using a 18.5G needle and twice-dialyzed against 4% sucrose. Adenovirus concentrations were measured at 10^{12} times the OD260 reading on a FLUOstar Omega plate reader (BMG Labtech, Offenburg, Germany). Adenovirus stocks were aliquoted and stored at -80ºC.

Production of recombinant vaccinia virus

Recombinant vaccinia virus expressing HCV-1 C-NS2 (rVV E12 C/B from Chiron Corporation) was obtained through the NIH AIDS Research and Reference Reagent Program[31,32]. rVV was amplified by infecting HeLa S3 cells in suspension culture at a multiplicity of infection of 0.5 for 48h. Intracellular virus was released by three freeze-thaw cycles and sonication. The virus was pelleted over a 36% sucrose cushion and resuspended in PBS[33].

HCV genome construction

Jc1FLAG2(p7Fluc2A) is a fully-infectious HCV reporter genome similar to Jc1FLAG2(p7nsGluc2A)[34]. This monocistronic genome encodes a Flag epitope, followed by a Gly-Ser-Gly-Ala linker, fused to the N-terminus of E2. The firefly luciferase (Fluc) reporter, in tandem with the foot and mouth disease virus autoproteolytic peptide sequence (2A), was inserted between p7 and NS2. Bi-nlsCre-JC1FLAG2 (HCV-CRE) was created by amplification of nlsCre-recombinase from a TRIP-based construct, followed by insertion into the MluI/PmeI sites of a wild-type or polymerase defective (GNN) BiYpet-JC1FLAG2[14] genome, replacing Ypet. nlsCre was similarly inserted into bicistronic versions of intergenotypic chimeras encoding the core-NS2 sequences of Con1 (1b), ED43 (4a), HK6a (6a) and QC69 (7a), which have been previously described[35]. All plasmid constructs were verified by DNA sequencing.

Gene		Primer sequence
CRE	+*	CCCAACGCGTATGCCCAAGAAGAAGAGGAAGGTGTCCA
	-	AGGGTTTAAACTTACTTGTACAGATCGCCATCTTC

* (+) forward primer; (-) reverse primer

HCV generation and infections

Huh-7.5.1 or Huh-7.5 cells were electroporated with in vitro transcribed full-length HCV RNA. 72h post-electroporation, the medium was replaced with DMEM containing 1.5% FBS and supernatants were harvested every six hours starting from 72h. Pooled supernatants were clarified by centrifugation at 1,500 xg, filtered through a 0.45 μm bottle top filter (Millipore, Billerica, MA) and concentrated using a stirred cell (Millipore). Viral titers ($TCID_{50}$) were determined using Huh-7.5 cells as previously described[28].

E2 antibody ELISAs

To determine end-point titers (EPT) of mouse sera, microplates (Corning Costar 3690) were coated with *Galanthus nivalis* lectin (GNL; Sigma) at 5 μg/ml overnight at 4°C. Microwells were washed four times with PBS containing 0.05% Tween 20 and blocked with non-fat milk (NFM; 4%, BioRad) diluted in the wash buffer. HCV glycoproteins E1 and E2 were produced by transient transfection of 293T cells with phCMV-H77 and solubilized in lysis buffer (25 mM Tris pH7.6, 140 mM NaCl, 1% Triton X-100, 0.5% NP-40 & 0.02% sodium azide). To adsorb E1E2, transfected cell lysate was diluted 1:30 in wash buffer containing NFM (1%) and incubated in the microwells for 1.5h at room temperature (RT). Following blocking and washing, serially diluted mouse sera were added to the ELISA plates and incubated at RT for 1h. Microwells were washed four times and incubated with peroxidase-conjugated goat anti-mouse IgG $F(ab')_2$ (diluted 1:2000) at RT for 1h. Following detection with tetramethylbenzidine substrate (TMB, Pierce), absorbance at 450 nm was measured with a micoplate reader (Molecular Devices). The EPT of each serum was defined as the reciprocal of the dilution giving a three-fold higher signal than the negative control. The positive control was a mixture of sera from 12 mice immunized with E2 and diluted at 1:2000; the negative control was serum from non-immunized Rosa26-Fluc mice. Due to high non-specific reactivity in mouse sera, only an EPT >200 is considered a true positive signal in this assay. Each ELISA was done in duplicate.

Histologic detection of HCV entry factors

Liver and spleen of mice injected with adenoviruses encoding human entry factors were harvested 24h post-injection and fixed using 4% paraformaldehyde. Tissue sections (8 μm) were deparaffinized and subjected to antigen retrieval by boiling for 30 min in citrate buffer (10mM sodium citrate, 0.05% Tween 20, pH 6.0). Entry factors were stained with human-specific primary antibodies for 16h at 4°C followed by secondary antibody staining using Alexa 488 or Alexa 633-conjugated antibodies for 2h at RT. For in situ detection of EGFP fluorescence, mouse tissue was immediately frozen in O.C.T. (Optimal Cutting Temperature) compound at -80°C. Tissue sections (~5-6 μm) were cut on poly-L-lysine coated slides. Secondary antibodies goat-anti-mouse or goat-anti-rabbit Alexa 488- or rhodamine-conjugates (Invitrogen; 1:1000) were used for immunofluorescence. Nuclei were detected using DAPI in VectaShield Mounting medium (Vector Laboratories, Burlingame, CA). Images were captured on an Axioplan 2 imaging fluorescence microscope (Zeiss, Thornwood, NY) using Metavue Software (Molecular Devices, Sunnyvale, CA). Images were processed using ImageJ software (NIH, Bethesda, MD).

Isolation of murine hepatocytes

Mice were anaesthetized by intraperitoneal injection of a mixture of 100mg/kg ketamine and 10mg/kg xylazine. Livers were perfused through the inferior vena cava for 5 minutes each with chelating buffer (0.5mM EGTA, 0.05M HEPES pH7.3 in Ca/Mg-free HBSS) at a flow rate of 2mL/min followed by collagenase solution (4.8mM $CaCl_2$, 100 U/mL Collagenase type IV, 0.05M HEPES pH7.3 in Ca/Mg-free HBSS). The resulting cell suspension was passed through a 100μm cell strainer, washed twice in HBSS and was fixed in 4% paraformaldehyde. Purity of isolated hepatocytes was over 90% in all preparations as confirmed by intracellular staining for murine albumin.

Western blotting

Perfused murine liver tissue was homogenized in lysis buffer containing 1% Triton X100, 50 mM Tris-HCl pH8, 150 mM NaCl, and Mini EDTA-free Protease Inhibitor Cocktail (Roche, Basel, Switzerland) for 30 min on ice. 15 μg of protein lysate was separated on 4-12% Bis/Tris NuPage polyacrylamide gels (Invitrogen). Proteins were transferred to nitrocellulose membranes and entry factors were detected using antibodies against CD81 (1:200), human SCARB1 (1:500), CLDN1 (1:200) or OCLN (1:200). β-actin (1:10000) was probed as a loading control. Following secondary antibody staining with HRP-conjugated anti-mouse IgG Fc (JIR, 1:10000), Western blots were visualized using SuperSignal West Pico (Thermo Scientific). 786O, 293T and HepG2 cell lysates, deficient in OCLN, CLDN1 and CD81, respectively, were used as negative controls. Huh-7.5 cell lysates served as positive control.

RT-PCR quantification of HCV entry factors and interferon-stimulated genes

To quantify expression of human and murine genes (entry factors and ISGs), the livers of FVB/NJ mice were harvested 24h post adenovirus injection. Total liver RNA was isolated using RNeasy isolation kit (Qiagen, Valencia, CA) and cDNA was synthesized from 0.5 μg RNA using a SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen, Carlsbad, CA) according to manufacturers instructions. Quantitative PCR was performed with a light cycler LC480 (Roche Applied Science, Indianapolis, IN) using an Applied Biosystems SYBR Green PCR Master Mix (Warrington, UK) and the following primer pairs:

Human Gene	Forward Primer	Reverse Primer
CD81	TGTTCTTGAGCACTGAGGTGGTC	TGGTGGATGATGACGCCAAC
SCARB1	CGGATTTGGCAGATGACAGG	GGGGGAGACTCTTCACACATTCTAC
CLDN1	CACCTCATCGTCTTCCAAGCAC	CCTGGGAGTGATAGCAATCTTTG
OCLN	CGGCAATGAAACAAAAGGCAG	GGCTATGGTTATGGCTATGGCTAC

Mouse Gene	Forward Primer	Reverse Primer
CD81	GGCTGTTCCTCAGTATGGTGGTAG	CCAAGGCTGTGGTGAAGACTTTC
SCARB1	CAAAAAGCATTTCTCCTGGCTG	AATCTGTCAAGGGCATCGGG
CLDN1	TTATGCCCCCAATGACAGCC	ATGAGGTGCCTGGAAGATGATG
OCLN	ACTAAGGAAGCGATGAAGCAGAAG	GCTCTTTGGAGGAAGCCTAAACTAC
GAPDH	ACGGCCGCATCTTCTTGTGCA	ACGGCCAAATCCGTTCACACC
viperin	TGCTGGCTGAGAATAGCATTAGG	GCTGAGTGCTGTTCCCATCT
IFI27	GCTTGTTGGGAACCCTGTTTG	GGATGGCATTTGTTGATGTGGAG
IFI44	AACTGACTGCTCGCAATAATGT	GTAACACAGCAATGCCTCTTGT
MX1	GACCATAGGGGTCTTGACCAA	AGACTTGCTCTTTCTGAAAAGCC
PKR	ATGCACGGAGTAGCCATTACG	TGACAATCCACCTTGTTTTCGT
2'OAS	ATGGAGCACGGACTCAGGA	TCACACACGACATTGACGGC
IFN-β	CAGCTCCAAGAAAGGACGAAC	GGCAGTGTAACTCTTCTGCAT
IP10	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTCAA

RT-PCR quantification of HCV RNA

Total RNA was isolated from mouse brain, liver and sera using the RNAeasy kit (Qiagen, Valencia, CA). HCV genome copy number was quantified by one step RT-PCR using Multicode-

RTx HCV RNA Kit Eragen (Madison, WI) and a light cycler LC480 (Roche Applied Science, Indianapolis, IN), according to manufacturers' instructions.

Bioluminescence Imaging
Unless otherwise specified, mice were injected with 10^{11} adenoviral PFU 24h prior to intravenous injection with $2x10^7$ $TCID_{50}$ HCV-CRE**.** At 72h post infection, mice were anaesthetized using ketamin/xylazine and injected intraperitoneally with 1.5 mg Luciferin (Caliper Lifesciences, Hopkinton, MA). Bioluminescence was measured using an IVIS Lumina II platform (Caliper Lifesciences).

In vitro neutralization assay
Serum of FVB/nJ mice, either mock immunized or immunized with rVV-HCV1 were collected and pooled 5 weeks following immunizations. Serial dilutions of mouse serum or monoclonal anti-HCV E2 antibody (clone 3/11) were pre-incubated with intergenotypic JFH1 chimeras expressing the structural proteins of genotypes 1a (H77), 1b (Con1 and J4), 2a (J6), 3a (S52), 4a (ED43), 5a (SA13), 6a (HK6a) or 7a (QC69) for one hour at 4°C. Supernatants were used to infect naïve Huh7.5 cells at a calculated multiplicity of infection of 0.1 for 6 hours after which cell were washed and medium was replaced. Cells were collected 48 hours post infection and were stained for expression of HCV NS5a and were analyzed by flow cytometry.

Flow Cytometry
Immune activation, depletion efficiency as well as the frequency of infected hepatocytes were confirmed by flow cytometry using an LSRII flow cytometer (BD Biosciences). For immune activation and depletion efficiency, PBMC and splenocytes of mice were isolated and purified via density gradient centrifugation. Cells were stained with directly fluorochrome-conjugated antibodies directed against CD3, CD4, CD8, CD11b and CD49b. For the determination of infection frequency, hepatocytes were isolated from Rosa26-GNZ mice, fixed in 4% paraformaldehyde, permeabilized in PBS + 0.01% Triton X-100 and stained with fluorochrome-conjugated antibodies against murine Albumin and CD81. Data were analyzed using Flowjo software (Treestar Software, Ashland, OR).

In vivo depletion
Mice were injected intraperitoneally with 100mg/kg of either anti-mouse Ly-6G, anti-mouse Asialo GM1 or a mixture of anti-mouse CD4 and CD8. Depletion of the cell populations was verified 24 hours post injection by flow cytometry. Adenoviral injection of HCV entry factor constructs was initiated following confirmation of depletion.

Statistical analysis
Statistical analyses were performed using Graphpad Prism Software (La Jolla, CA). Statistics were calculated using Kruskal-Wallis one-way analysis of variance. P values below 0.05 were considered statistically significant.

References:

28 Lindenbach, B. D. *et al.* Complete Replication of Hepatitis C Virus in Cell Culture. *Science* **309**, 623-626 (2005).
29 Flint, M. *et al.* Characterization of hepatitis C virus E2 glycoprotein interaction with a putative cellular receptor, CD81. *J Virol* **73**, 6235-6244 (1999).
30 Schoggins, J. W., Gall, J. G. & Falck-Pedersen, E. Subgroup B and F fiber chimeras eliminate normal adenovirus type 5 vector transduction in vitro and in vivo. *J Virol* **77**, 1039-1048 (2003).

31 Selby, M. *et al.* Hepatitis C virus envelope glycoprotein E1 originates in the endoplasmic reticulum and requires cytoplasmic processing for presentation by class I MHC molecules. *J Immunol* **162**, 669-676 (1999).
32 Cooper, S. *et al.* Analysis of a successful immune response against hepatitis C virus. *Immunity* **10**, 439-449 (1999).
33 Law, M. & Smith, G. L. in *Vaccinia Virus and Poxvirology Methods and Protocols Methods in Molecular Biology Series* (ed S.N. Isaacs) 187-204 (Humana Press, 2004).
34 Marukian, S. *et al.* Cell culture-produced hepatitis C virus does not infect peripheral blood mononuclear cells. *Hepatology* **48**, 1843-1850 (2008).
35 Gottwein, J. M. *et al.* Development and characterization of hepatitis C virus genotype 1-7 cell culture systems: role of CD81 and scavenger receptor class B type I and effect of antiviral drugs. *Hepatology* **49**, 364-377 (2009).